Original

Genomic Characterisation of Virulence and In Vivo Pathogenicity of Azole-Antifungal Resistant *Candida* from HIV/AIDS Patients in Southeast Nigeria

[1]Irene Ifeyinwa Eze, [1]Edmund Ndudi Ossai, [1]Lawrence Ulu Ogbonnaya, [2]Akinwale Michael Efunshile, [3]Chito Clare Ekwealor, [3]Christie Amechi Oyeka

[1]Department of Community Medicine, College of Medicine, Alex Ekwueme Federal University, Ndufu-Alike Abakaliki, Ebonyi State, Nigeria.
[2]Department of Medical Microbiology and Parasitology, College of Medicine, Alex Ekwueme Federal University, Ndufu-Alike Abakaliki, Ebonyi State, Nigeria.
[3]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Corresponding author: Irene Ifeyinwa Eze, Department of Community Medicine, College of Medicine, Alex Ekwueme Federal University, Ndufu-Alike Abakaliki, Ebonyi State, Nigeria. jorenebiz@yahoo.com:+2348032653216

Article history: Received 24 August 2025, Reviewed 16 November 2025, Accepted for publication 08 December 2025

ABSTRACT

Background: Oral candidiasis remains a common opportunistic infection among people living with HIV/AIDS, especially in low-resource settings. Rising antifungal resistance and systemic candidiasis complicate treatment. This study investigated the genomic features and virulence mechanisms of *Candida* species isolated from HIV-positive individuals in southeast Nigeria.

Methods: Oral swabs (n=514) from HIV patients were collected and cultured on Chromogenic agar for *Candida,* with azole susceptibility assessed through the Kirby-Bauer disk-diffusion method. Representative isolates underwent whole-genome sequencing to identify virulence genes and mechanisms. A pathogenicity study was conducted on 16 rats, divided into four groups: *Candida albicans*, *Candida glabrata*, a mixed inoculum, and a non-inoculated control. Over 28 days, morbidity, mortality, weight changes, and organ infections were evaluated.

Results: All sequenced strains belonged to Sequence Type 15 with conserved genomes. Key virulence genes (*waaF*, *waaG*, *pilH*, and *pvdS)* and housekeeping genes (*argS, gyrB, ileS, nuoC, ppsA, recA, rpoB, rpoD*) were consistently expressed, supporting biofilm formation, stress tolerance, immune evasion, and persistence mechanisms. In vivo, rats inoculated with *C. albicans* or mixed inoculum showed severe morbidity, high mortality (100% and 75% respectively), weight loss, and tissue necrosis, indicating systemic infection.

Conclusion: Azole-resistant *Candida* species from HIV-infected individuals exhibit diverse genetic and phenotypic mechanisms that drive virulence and resistance. The findings emphasise their pathogenic potential and the challenges they pose for diagnosis and treatment. Genomic similarity among isolates indicates clonal expansion and potential interspecies gene exchange, underscoring the importance of integrated antifungal surveillance and stewardship in resource-limited settings.

Keywords: Occupational health, industrial noise, tinnitus, hearing conservation, mixed methods review

How to cite this article

Eze II, Ossai EN, Ogbonnaya LU, Efunshile AM, Ekwealor CC, Oyeka CA. Genomic Characterization of Virulence and In Vivo Pathogenicity of Azole-Antifungal Resistant Candida from HIV/AIDS Patients in Southeast Nigeria. The Nigerian Health Journal 2025; 25(4):1420 – 1432.
https://doi.org/10.71637/tnhj.v25i4.1193

INTRODUCTION

Oral candidiasis (OC) is a prevalent opportunistic infection among people living with HIV (PLHIV), especially those with advanced immunosuppression[1], often resulting in poor nutrition and severe systemic complications. [2,3] While *Candida albicans* is the leading cause, non-*albicans Candida* species such as *C. glabrata*, *C. tropicalis*, and *C. krusei* are increasingly reported, frequently showing reduced susceptibility to azole antifungals.[4,5]

Candida pathogenicity is linked to virulence factors and genes that drive adhesion, biofilm formation, immune evasion, and drug tolerance, particularly in immunocompromised hosts.[2,6] Resistance-associated changes, including mutations in genes like ERG11, efflux pumps activation and transcriptional regulators, can further enhance virulence,[7] complicating treatment.[8] Despite advancements globally, data on the interplay between resistance and virulence gene expression in *Candida* species from resource-limited settings are scarce.[9] In Nigeria and much of sub-Saharan Africa, molecular testing for OC in PLHIV is rare and antifungal susceptibility testing is not routinely performed,[10] resulting in empirical treatment and fostering resistance.[9]

Comprehensive approaches such as whole-genome sequencing (WGS) offer powerful tools for identifying virulence genes, resistance mechanisms, and isolate relationships. Integrating WGS with in vivo animal models enables simultaneous characterisation of mechanisms, pathogenic and resistance profiles, and the generation of data that can directly inform clinical management, antifungal stewardship, and surveillance strategies.

This study, utilising WGS and animal models, aimed to (i) identify and characterise virulence genes in azole-resistant *Candida* from HIV patients, (ii) detect molecular mechanisms linked to virulence traits, and (iii) determine in vivo virulence potential using animal models.

The research will fill key knowledge gaps in Nigeria, supporting better clinical management and surveillance.

METHODOLOGY

Study area, study design and population: The study was conducted in Ebonyi State, southeast Nigeria. A health facility-based cross-sectional study design was conducted at health facilities providing comprehensive HIV services, comprising voluntary and confidential counselling and testing, prevention of HIV transmission, prophylaxis, diagnosis and treatment of HIV-related conditions, including opportunistic infections.[9] Two hospitals providing comprehensive HIV services were purposively selected based on their geographical location (urban and rural), hospital type (public and private), and HIV patient load (high and low). Alex-Ekwueme Federal University Teaching Hospital, Abakaliki (AEFUTHA), is a public hospital located in the urban area with the highest patient load (1,974). St. Patrick's Mile Four Hospital, Abakaliki, is a private/missionary hospital situated in a rural area with the highest patient load (1,333). Individuals on antifungal drugs, immunosuppressants and antibiotics within six months prior to study were excluded.

Sampling technique: A minimum sample size of 514 was determined using the single-proportion formula. Sample size was allocated proportionally to the two selected hospitals based on their enrollees in HIV care.

A systematic random sampling method was employed to select study participants using the hospital attendance register as the sampling frame. The sampling interval (k) was calculated as $k = N/n$, where N is the average number of patients attending the clinic daily, and n is the number of participants targeted for recruitment each day. On each clinic day, the first participant was randomly selected by ballot, followed by every kth patient until the daily sample size was reached.

Ethical Considerations: Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the Alex-Ekwueme Federal University Teaching Hospital, Abakaliki (AEFUTHA) (AEFUTHA/REC/Vol 3/2023/225), Nigeria and St. Patrick's Mile Four Hospital, Abakaliki (RE/M4H/100/23). Permission was obtained from the management of the HIV clinics at the hospitals. Informed written consent for participation and publication of the findings was obtained from patients (aged 18 years and above) by having them sign the consent form after a thorough explanation of the study's objectives, risks, and benefits. For patients under 18, informed written consent was obtained from their parents, and assent from the patients. Patients were informed that participation is voluntary, and they are free to withdraw from the research at any time without consequences. Furthermore, patients were assured that the information and laboratory findings provided would

Print ISSN: 0189-9287 Online ISSN: 2992-345X

be kept confidential and that personal identifiers would be coded to ensure anonymity.

Data Collection and Analysis

Identification of azole-resistant *Candida* species

Oral swabs were collected by trained research assistants and analysed for microbes. *Candida* species were cultured on Chromogenic agar (CHROMOagar Company, Italy), a selective and differential medium, following the manufacturer's instructions.[12]

Azole-antifungal susceptibility of the isolated *Candida* species was assessed using the Kirby-Bauer agar disk diffusion method,[13] with inhibition zones measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[14]

Molecular analysis - Whole Genome Sequencing

Three representative resistant isolates underwent whole-genome sequencing and bioinformatics analysis to characterise resistance profiles, identify virulence genes, and classify resistance mechanisms. The process included DNA extraction, read processing, genome assembly, and genome annotation.

Genomic DNA Extraction: DNA was extracted using the Zymo Research Fungal/Bacterial DNA kit. One mL of the fungal isolate contained in Sabouraud dextrose broth was transferred into a ZR BashingBead™ Lysis Tube, mixed with 750 µL of Lysis Solution, and vortexed at maximum speed for 10 minutes. Debris was removed by centrifuging at 10,000 × g for 1 minute. After centrifugation at 10,000 × g for 1 minute, 400 µL of supernatant was mixed with 1,200 µL of DNA Binding Buffer and applied to Zymo-Spin™ Column. The lysate was washed by repeated addition of 200 µL of DNA Pre-Wash Buffer to the column and centrifuging at 10,000 × g for 1 minute. Thereafter, 50 µL of nuclease-free water was added directly to the column matrix, allowed for 2 minutes at room temperature and centrifuged at 10,000 × g for 30 seconds to elute the DNA. DNA quality was assessed using NanoDrop spectrophotometer.[15]

DNA Fragmentation: Extracted DNA was fragmented and adapter-tagged in a single-step reaction using Tn5 transposase. Limited-cycle PCR amplified the fragments and incorporated index sequences using DreamTaq Green PCR Master Mix (2X; ThermoFisher Scientific). Libraries were quantified, pooled and sequenced on the MiSeq Illumina platform, generating 2 × 300 bp paired-end reads.[16]

Reads Processing and Genome Assembly: Raw reads underwent quality control with CLC Genomics Workbench (v11.0.1) (Qiagen Bioinformatics, Aarhus, Denmark) and trimming with cutadapt (v.2.2). De novo genome assembly used Unicycler v0.4.8 with default parameters optimised for fungal genomes. Two rounds of polishing with Pilon 1.23 refined the draft assemblies, with the resulting contigs evaluated for quality metrics such as N50, total assembly size, and coverage depth using QUAST.[17-19]

Genome Annotation and Profiling: Annotation of the three *Candida species* genomes and 15 additional strains downloaded from the NCBI database was performed using the companion fungi annotation pipeline. Resistance genes, virulence genes, and their mechanisms were characterised using a combined BLASTP and k-mer-based approach. This analysis systematically classified resistance virulence genes by mechanisms using specialised gene repositories, including VFDB and CARD.[20-22]

Virulence assessment in an animal model

Virulence assessment in laboratory animals was conducted using immunosuppressed rats infected with *Candida* species, with morbidity, mortality, organ fungal burden, and histopathology as primary outcomes.

Animals and immunosuppression: Sixteen six-week-old female albino rats, obtained from the Biochemistry Department animal house, University of Nigeria, Nsukka, were housed under a 12 h/12 h light-dark cycle in well-ventilated cages with free access to food and water for a 7-day acclimatisation period.

On acclimatisation day 4 (three days before *Candida* inoculation*)*, rats received cyclophosphamide (150mg/kg intraperitoneally to induce neutropenia, followed by maintenance doses of 100mg/kg on days 7, 14 and 21 to sustain the immunosuppression.[23]

Preparation of *Candida* inoculum: The McFarland Standard was prepared by mixing 0.6 ml of a 1% barium chloride with 99.4ml of 1% sulphuric acid and sealing the mixture to prevent evaporation. Pure *Candida* cultures (0.1g) were suspended in normal saline, and the turbidity was adjusted to match the McFarland Standard, with absorbance checked to ensure consistency.

Print ISSN: 0189-9287 Online ISSN: 2992-345X

***Candida* inoculation protocol:** Three days after induction of immunosuppression, rats were randomly assigned to four groups (n = 4 per group) Each rat received a 0.20 ml tail-vein injection of *Candida* suspension (5x10-6 CFU ml-1)[24] as follows: Group 1- *C. albicans,* Group 2 - *C. glabrata*, Group 3 - a mixed *C. albicans and C. glabrata,* and Group 4 (control) – oral normal saline (50ml/kg/day). All animals were monitored for 28 days post-inoculation.

Monitoring and study endpoint: Rats were examined daily for signs of morbidity (weakness, immobility, reduced feed and water intake) and mortality, and body weights were recorded on days 2, 7, 14, 21 and 28. Survival was recorded, and animals reaching a moribund state were humanely euthanised.

Post-mortem analysis: At euthanasia or death, the kidneys, liver, and spleen were collected and homogenised for microbiological and histological analysis.
For microbiology, homogenates were serially diluted and plated on Sabouraud dextrose agar containing tetramycin to inhibit bacterial growth, then incubated at 37 ^{0}C for 72 hours, with *Candida* confirmed by microscopy of budding yeast cells and characteristic, purple-stained oval shapes with crystal violet.
For histological, organ sections were examined for tissue damage and evidence of fungal dissemination.

Statistical analysis
Descriptive statistics were performed using International Business Machine, Statistical Product and Service Solutions (IBM-SPSS) software (ver. 25.0; IBM Corp., Armonk, NY, USA) and presented as frequencies and proportions for categorical variables and as means and standard deviations for continuous variables.
Genomic analysis of virulence genes and mechanisms of resistance across *Candida sp.* isolates was performed using Tableau[25] for quantitative analysis, ClusVis for clustering and dimensionality reduction, and Python for correlation analysis and heatmap generation, to reveal conserved and distinct gene profiles linked to specific mechanisms of resistance and virulence.[26,27]

RESULTS

The socio-demographic and clinical profile of the 514 participants showed that most participants were female (369, 71.8%), married (354, 68.9%), had completed secondary education (194, 37.7.%), and were self-employed (210, 40.9%). The mean age was 38.3 ± 13.1 years, the mean weight was 59.7 ± 17.5 kg, and the average duration on highly active anti-retroviral therapy (HAART) was 8.6 ± 5.1 years.

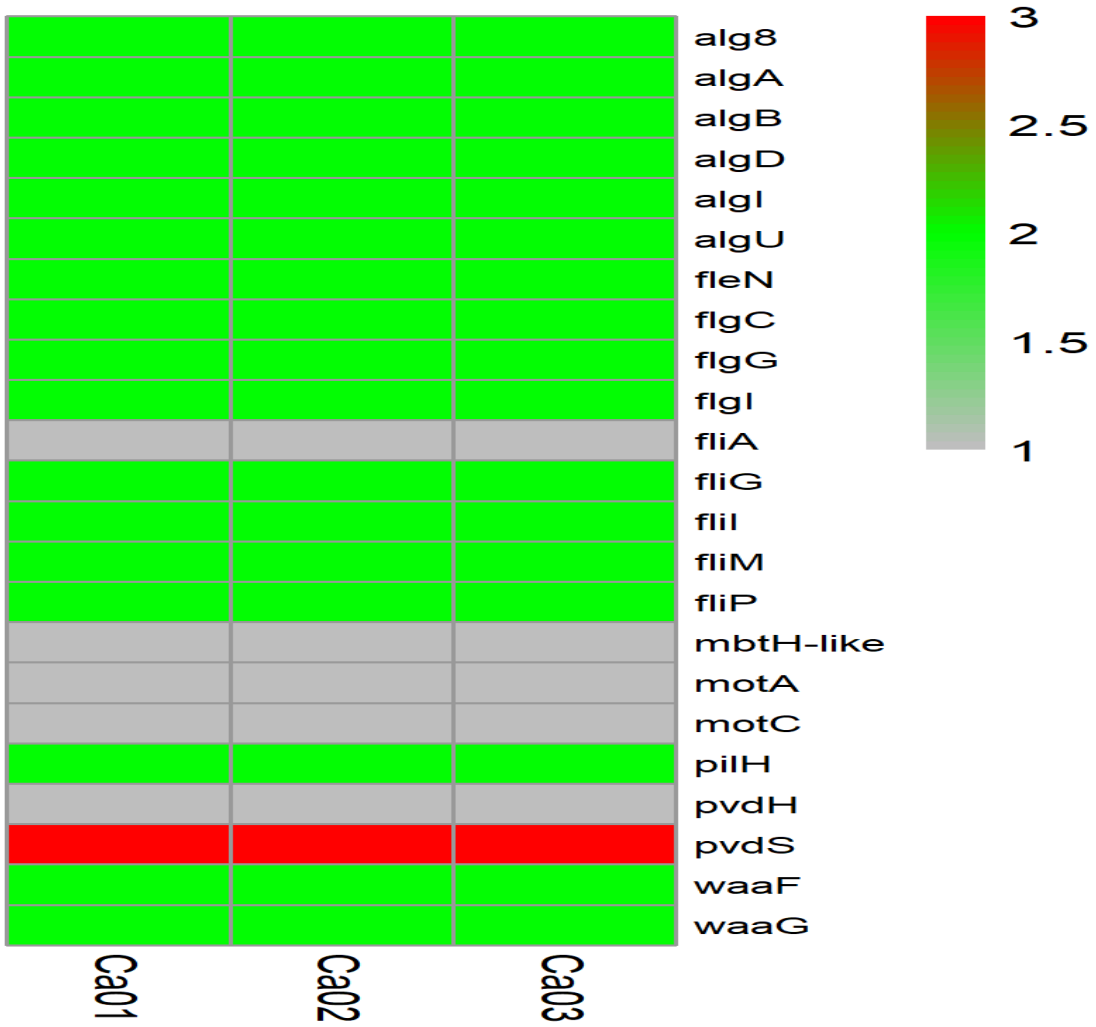
Common oral lesion presentations included white tongue patches (28, 53.0%), mouth pain (12, 23%), loss of taste (8, 15.0%), and difficulty swallowing (5, 9.0%). *Candida* was isolated in 195 participants (37.9%), with *C. albicans* (99, 19.3%) as the most frequent species, followed by *C. glabrata* (64, 12.5%), *and C. krusei* (26, 5.1%). *C. tropicalis* was the least (6, 1.2%), while mixed infections involving *C. albicans* and non-*albicans* species occurred in four cases (0.8%)

Azole antifungal susceptibility testing revealed very high resistance (99.5%) to fluconazole, ketoconazole, itraconazole, and voriconazole. In contrast, clotrimazole resistance was markedly lower (25.6%). Species-specific analysis showed complete resistance (100%) of *Candida albicans, C. krusei,* and *C. tropicalis* to the four major azoles, while *C glabrata* exhibited 98.4% resistance. Resistance to clotrimazole varied across species, with *C. glabrata* (46.2%), *C. tropicalis* (33.3%), *C. krusei* (29.6%), and *C. albicans* (10.9%) showing the highest rates.

Figure 1 shows a Heatmap of Virulence-Associated Genes. The heatmap analysis of *Candida* species isolates (*C. glabrata, C. albicans,* and *C. krusei*) highlights the distribution and presence of virulence-associated genes critical to pathogenicity. Genes such as alg8**,** algA**,** algB**,** algD**,** and algI**,** are uniformly expressed across all isolates. Similarly, there are waaF and waaG**,** involved in the biosynthesis of cell wall components. The siderophore synthesis gene pvdS is prominently represented.

Motility-associated genes such as fliA**,** fliG**,** and fliM are consistently present, as well as the expression of pilH**,** linked to surface adherence and twitching motility. Similarly, motA and motC**,** typically associated with flagellar function, are less prominent. Interestingly, some genes, such as mbtH-like**,** are absent or show no detectable expression.

Print ISSN: 0189-9287 Online ISSN: 2992-345X



C. glabrata (Ca01), *C. albicans* (Ca02), and *C. krusei* (Ca03)

Figure 1: Heatmap of Virulence-Associated Genes

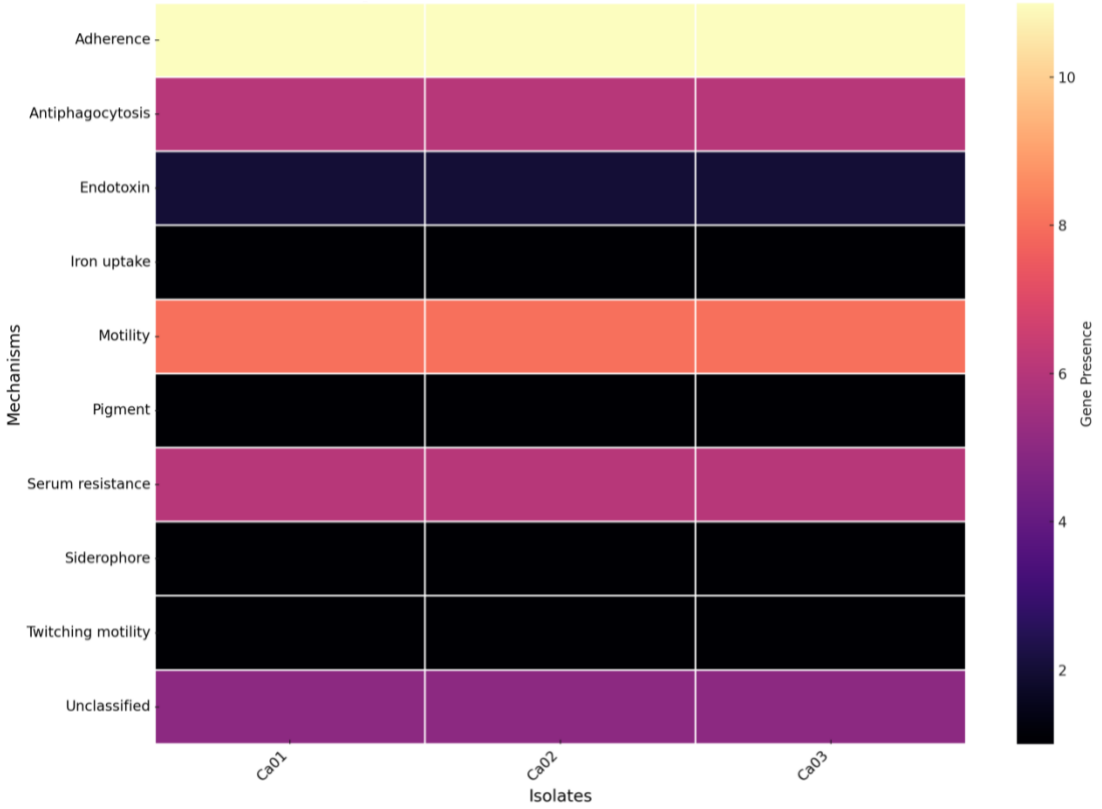
Figure 2 shows the Heatmap of virulence mechanisms across *Candida* isolates. The analysis of virulence mechanisms reveals highly conserved profiles across all measured categories.

Adherence mechanisms are the most abundant category with 11 counts per isolate. **Motility,** represented by eight counts per isolate, is another dominant virulence mechanism. There is **anti-phagocytosis** and **serum resistance,** with six counts per isolate, respectively observed.

Additionally, there is the **unclassified category,** with five counts per isolate, representing a potential novel virulence pathway that has yet to be characterised.

Endotoxin production, though less frequent (two counts per isolate), was also identified, as well as mechanisms associated with **iron uptake, siderophore production,** and **pigment synthesis,** each with one count per isolate.

Print ISSN: 0189-9287 Online ISSN: 2992-345X



C. glabrata (Ca01), *C. albicans* (Ca02), and *C. krusei* (Ca03)

Figure 2: Heatmap of Virulence Mechanisms Across Candida Isolates

Figure 3 shows the Heatmap of Virulence-Associated Factors Across Genes. The heatmap highlights the distribution of virulence-associated factors across various genes, revealing a multifaceted genetic basis of pathogenicity. **pilH** and **waaG** show significant contributions to antiphagocytosis and serum resistance. Similarly, **algD, algE,** and **algG** are primarily associated with adherence and motility. The siderophore production pathway and iron acquisition are strongly linked to **pvdS,** while **motC, flhA,** and **motB** are linked to motility.

Additionally, **fimG, fimH,** and **fimI** play key roles in adherence to host cells and biofilm formation. Genes such as **waaF** further enhance resistance to serum-mediated killing through LPS core biosynthesis. **pvdS** and **mbtH-like** regulate siderophore synthesis, ensuring efficient iron uptake. Unclassified factors are associated with diverse genes, such as **mbtH-like.**

Print ISSN: 0189-9287 Online ISSN: 2992-345X

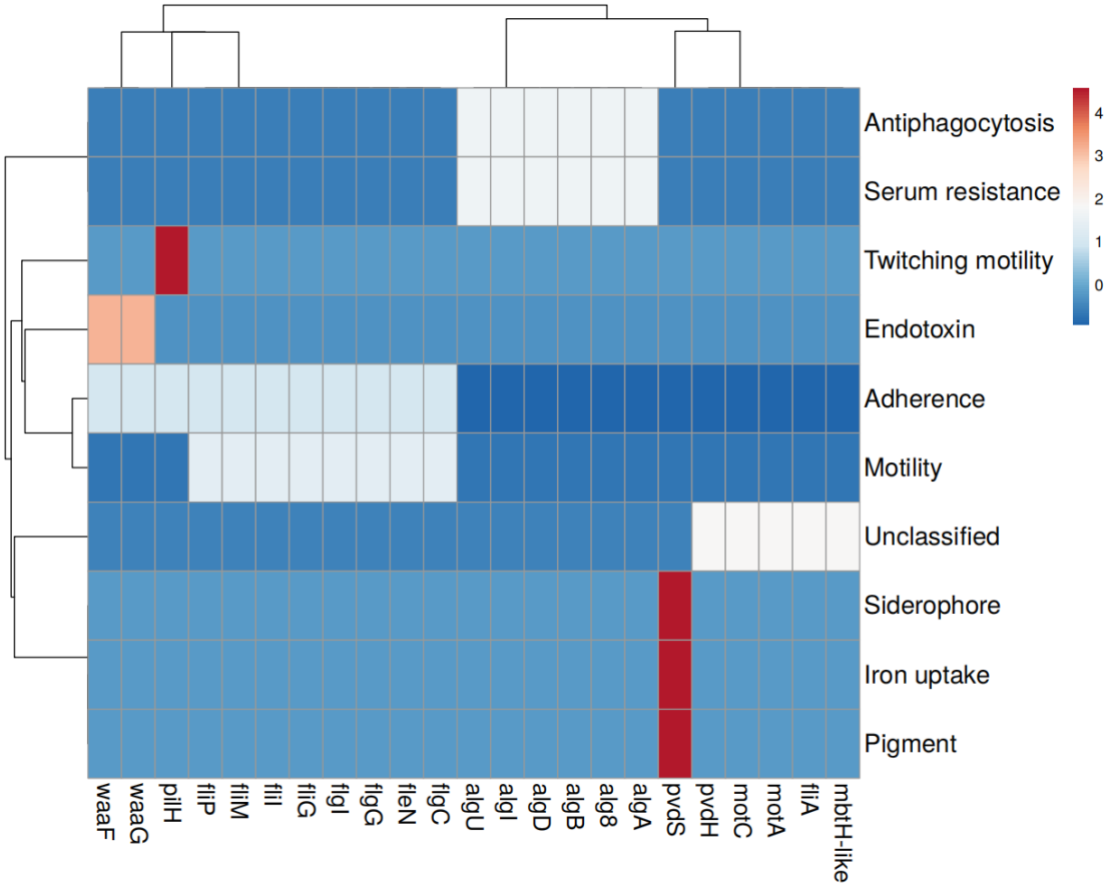


Figure 3: Heatmap of Virulence-Associated Factors Across Genes

Table 1 shows the Multilocus Sequence Typing (MLST) analysis of three *Candida* species- *C. glabrata* (Ca01), *C. albicans* (Ca02), and *C. krusei* (Ca03) genomes, all belonging to Sequence Type 15 (ST15), sharing identical allelic at eight housekeeping genes (argS, gyrB, ileS, nuoC, ppsA, recA, rpoB, and rpoD).

The **aminoacyl-tRNA synthetase** genes **argS** and **ileS** support protein synthesis and stress adaptation, while **gyrB is required for** DNA supercoiling and replication, with mutations linked to reduced susceptibility to DNA-targeting agents.

The mitochondrial respiratory gene **nuoC** is associated with biofilm formation and antifungal tolerance, and **ppsA promotes** metabolic flexibility in nutrient-limited conditions.

The DNA repair gene **recA** enhances genomic stability and may counter antifungal-induced damage, where **rpoB** and **rpoD encode RNA polymerase subunits, adjusting transcription** in response to stresses to sustain survival and virulence.

Print ISSN: 0189-9287 Online ISSN: 2992-345X

Table 1: Multilocus Sequence Typing (MLST) analysis of the three *Candida* species genomes

Genome File	Species	Sequence Type (ST)	argS (Allele)	gyrB (Allele)	ileS (Allele)	nuoC (Allele)	ppsA (Allele)	recA (Allele)	rpoB (Allele)	rpoD (Allele)
Ca01.fasta	*Candida albicans*	15	argS (11) - Protein synthesis, stress response	gyrB (24) - DNA replication, antifungal resistance	ileS (20) - Protein translation, stress tolerance	nuoC (15) - Biofilm formation, energy production	ppsA (20) - Metabolic flexibility, nutrient adaptation	recA (33) - DNA repair, antifungal resilience	rpoB (7) - Transcription stability, immune evasion	rpoD (28) - Transcription regulation, stress adaptation
Ca02.fasta	*Candida glabrata*	15	argS (11) - Protein synthesis, stress response	gyrB (24) - DNA replication, antifungal resistance	ileS (20) - Protein translation, stress tolerance	nuoC (15) - Biofilm formation, energy production	ppsA (20) - Metabolic flexibility, nutrient adaptation	recA (33) - DNA repair, antifungal resilience	rpoB (7) - Transcription stability, immune evasion	rpoD (28) - Transcription regulation, stress adaptation
Ca03.fasta	*Candida krusei*	15	argS (11) - Protein synthesis, stress response	gyrB (24) - DNA replication, antifungal resistance	ileS (20) - Protein translation, stress tolerance	nuoC (15) - Biofilm formation, energy production	ppsA (20) - Metabolic flexibility, nutrient adaptation	recA (33) - DNA repair, antifungal resilience	rpoB (7) - Transcription stability, immune evasion	rpoD (28) - Transcription regulation, stress adaptation

Print ISSN: 0189-9287 Online ISSN: 2992-345X

Table 2 shows the in vivo evaluation of the virulence/pathogenicity of azole-resistant *Candida* species in laboratory animals (rats). There was consistent weight loss in all three groups of laboratory animals inoculated with *Candida* species- *Candida albicans* (Ca-G1), *Candida glabrata* **(Cg-G2),** and a mixed inoculum of both species **(Ca/Cg-G3).** However, the control group showed a steady weight increase. Additionally, mortality was recorded in the group of laboratory animals infected with *C. albicans* (4, 100%) and the group infected with a mixed culture of *C. albicans* and *C. glabrata* (3, 75%). However, there were no records of death of the laboratory animals infected with only *C. glabrata* (although they exhibited consistent **weight decline)** and among the non-inoculated control group.

Macroscopic examination of harvested tissues from laboratory animals revealed tissue necrosis, and microbiological tissue cultures showed budding yeast on wet preparation and purple-stained oval cells with crystal violet stain, as shown in Figure 4

Table 2: Weights measurements of animals (rats) during in vivo assessment of virulence of azole-resistant *Candida*

Rat's code	**Weight of rats during seven days acclimatization**		**Weight of rats after inoculation with microorganisms**				
	1st	**7th**	**Day 2**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group1							
LE	130g	163g	160g*	-	-	-	-
RL	145g	168g	165g*	-	-	-	-
RH	138g	170g	168g	165g*	-	-	-
LH	98g	155g	153g	155g	150g*	-	-
Group2							
RL	140g	170g	168g	165g	160g	160g	158g
LL	135g	160g	158g	158g	154g	151g	150g
RH	138g	165g	160g	158g	155g	153g	150g
LH	129g	160g	157g	153g	152g	150g	150g
Group3							
RL	135g	162g	160g	155g*	-	-	-
LL	138g	168g	165g	160g	157g	157g	155g
RH	136g	162g	160g*	-	-	-	-
LH	132g	165g	163g	163g	162g*	-	-
Group4	Day1	Day7					
RL	110g	175g	178g	181g	185g	190g	196g
LL	87g	150g	153g	156g	160g	161g	163g
RH	150g	200g	200g	205g	209g	211g	215g
LH	143g	170g	178g	181g	189g	193g	197g

Group 1 - Candida albicans (Ca-1), *Group 2 - Candida glabrata* (**Cg-2)**, *Group 3* -mixed inoculum of *Candida albicans* and *Candida glabrata (***Ca/Cg-3),** *Group 4* - Non-inoculated control group
RL -Right leg, LL -Left leg, RH -Right hand, LH- Left hand
***** Mortality (Moribundity or/and death of rat

Print ISSN: 0189-9287 Online ISSN: 2992-345X

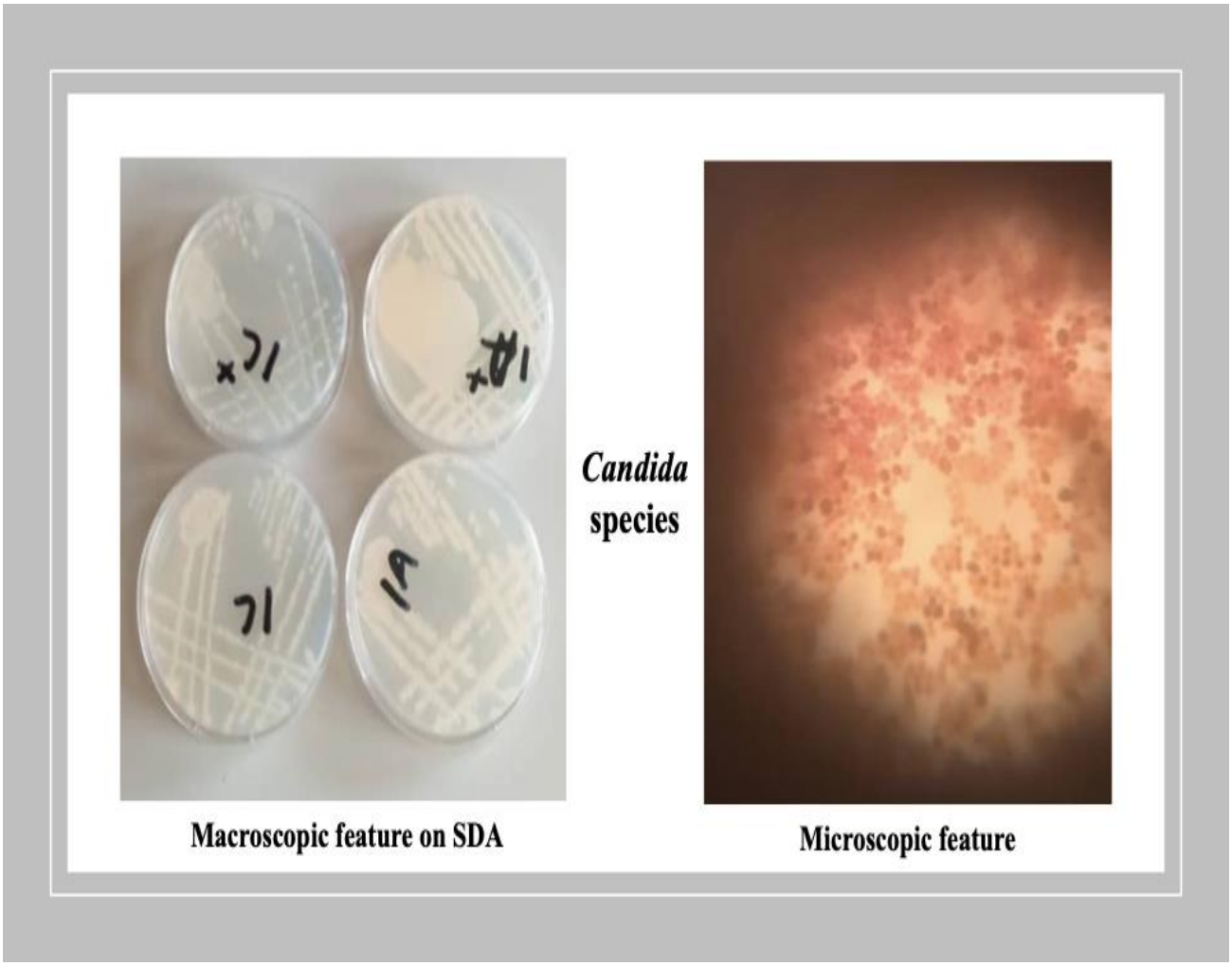


Figure 4: Macroscopic and Microscopic features of tissue cultures from Azole-resistant *Candida*-infected rat

Print ISSN: 0189-9287 Online ISSN: 2992-345X

DISCUSSION

This study integrates genomic and in vivo data on azole-resistant *Candida* isolates from HIV-positive individuals, showing conserved virulence strategies, clonal relatedness, and high pathogenic potential. Sequencing revealed a conserved genomic type (ST15), with identical allelic in eight housekeeping genes (*argS, gyrB, ileS, nuoC, ppsA, recA, rpoB, rpoD*) that support stress tolerance, metabolic plasticity, DNA repair, and transcriptional adaptation, promoting persistence under antifungal pressure. [28,29]

The ide ST15, previously linked to invasive infections, suggests clonal spread of a lineage with selective advantages with biofilm formation, drug resistance, and immune evasion.[28] Beyond multilocus sequence typing, functional data showed a conserved virulence gene set, including adherence genes (*pilH, fimG, fimH*), alginate biosynthesis (*alg8, algA, algB, algD, algI*), and lipopolysaccharide-core assembly genes (*waaF, waaG*), all linked to biofilm development, immune evasion, and persistence, hallmarks of chronic and drug-resistant infections.[30]

Biofilms composed of cells embedded in an extracellular matrix display high antifungal tolerance through efflux pump upregulation, reduced ergosterol synthesis, limited drug penetration, quiescent persistent cells, and altered sterol composition and membrane dynamics.[31] These features create physical and functional barriers to intracellular drug action, contributing to treatment failure in mucosal candidiasis.[31]

Additional virulence mechanisms included secreted hydrolytic enzymes (aspartyl proteases and phospholipases) that damage,[32] siderophore-mediated iron uptake regulated by *pvdS*[33] and motility-related genes (*fliA, fliG, fliM*), though uncommon in fungi, that likely promote hyphal extension and epithelial penetration, facilitating invasion and dissemination.[34] Morphogenetic switching, particularly the yeast-to-hyphal transition, remains a hallmark of *C. albicans* virulence,[32,35] and the presence of poorly characterised genes suggests further unrecognised virulence pathways. The rat model corroborated these genomic insights: *C. albicans* or mixed *C. albicans*–*C. glabrata* infections caused marked morbidity, mortality, weight loss, and dissemination to the kidney, liver, and spleen, confirming the invasive capacity of these isolates. The synergistic effect of co-infection underscores the clinical significance of mixed-species candidiasis.[34] However, *C. glabrata* alone produced only mild weight loss without death, reflecting its lower intrinsic virulence but strong biofilm-associated persistence.[36]

Overall, azole-resistant *Candida* isolates shared a stable clonal lineage enriched for virulence determinants with major implications for HIV/AIDS care, particularly in mixed-species infections, and point antifungal strategies targeting adhesion, biofilm formation, and iron acquisition.

A key limitation was the lack of detailed histopathology, which would characterise tissue invasion and organ involvement; only macroscopic damage and lethal infection were documented. Future work should include histologic, genomic, and transcriptomic analyses of unclassified virulence factors to identify new therapeutic targets.

Contributions to Knowledge

This study has made the following significant contributions to scientific knowledge:

1. Uncovered the role of regulatory mechanisms in phenotypic variation: Despite genetic similarity, variations in resistance and virulence were observed, suggesting regulatory or post-transcriptional influences beyond sequence mutations.
2. Reported atypical resistance genes in *Candida* genomes: The detection of genes like *blaKPC* and bacterial genes suggests potential inter-kingdom gene transfer and highlights the need for expanded surveillance of resistance genes.
3. Highlighted conserved virulence mechanisms across species: The shared presence of virulence genes among diverse *Candida* species indicates evolutionary conservation of traits crucial for survival in immunocompromised hosts.
4. Validated animal weight as a marker of disease progression: This demonstrated that weight loss in animal models correlates with infection severity, supporting its utility in evaluating antifungal efficacy.

CONCLUSION

Azole-resistant *Candida* isolates exhibited a conserved ST15 genome architecture, key virulence genes for adherence, biofilm formation, and immune evasion, and were virulent in an animal model. These findings support further functional genomics and anti-virulence studies, including the exploration of regulatory

Print ISSN: 0189-9287 Online ISSN: 2992-345X

mechanisms and uncharacterised resistance genes, and highlight the value of simple, non-invasive metrics, such as body weight, for monitoring disease progression and treatment responses in antifungal drug development.

DECLARATIONS

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements

We appreciate the management and staff of AEFUTHA Molecular Laboratory for permitting us to use their molecular laboratory and facilities.

List of Abbreviations

AEFUTHA - Alex Ekwueme Federal University Teaching Hospital Abakaliki
AIDS - Acquired Immunodeficiency Syndrome
CLSI – Clinical Laboratory Standard Institute
HAART - Highly Active Antiretroviral Therapy
HIV- Human Immunodeficiency Virus
HREC – Health Research Ethics Committee
OC – Oral Candidiasis
PLHIV – People Living with HIV
ST - Sequence Type
WGS – Whole Genome Sequencing

References

1. Suryana K, Suharsono H, Antara IG. Factors associated with oral candidiasis in people living with HIV/AIDS: a case control study. HIV/AIDS-Research and Palliative Care. 2020:33-9. https://doi.org/10.2147/HIV.S236304
2. Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA. Oral candidiasis: A disease of opportunity. Journal of fungi. 2020;6(1):15. https://doi.org/10.3390/jof6010015
3. Taylor, M., Brizuela, M., and Raja, A.) Oral candidiasis. In: StatPearls. Treasure Island (FL): Stat Pearls Publishing 2023.
4. Bilal H, Shafiq M, Hou B, Islam R, Khan MN, Khan RU, Zeng Y. Distribution and antifungal susceptibility pattern of Candida species from mainland China: a systematic analysis. Virulence. 2022;13(1):1573-89.
5. Ekwealor CC, Nweke CJ, Anaukwu CG, Anakwenze VN, Ogbukagu CM, Mba AN. Prevalence and antifungal susceptibility pattern of oral candidiasis among HIV-infected patients in a Mission Hospital, southeast Nigeria. African Journal of Clinical and Experimental Microbiology. 2023;24(3):289-98.
6. Rodríguez-Cerdeira C, Gregorio MC, Molares-Vila A, López-Barcenas A, Fabbrocini G, Bardhi B, Sinani A, Sánchez-Blanco E, Arenas-Guzmán R, Hernandez-Castro R. Biofilms and vulvovaginal candidiasis. Colloids and Surfaces B: Biointerfaces. 2019;174:110-25.
7. Wall G, Montelongo-Jauregui D, Bonifacio BV, Lopez-Ribot JL, Uppuluri P. Candida albicans biofilm growth and dispersal: contributions to pathogenesis. Current opinion in microbiology. 2019 Dec 1;52:1-6.
8. Rolo J, Faria-Gonçalves P, Barata T, Oliveira AS, Gaspar C, Ferreira SS, Palmeira-de-Oliveira R, Martinez-de-Oliveira J, Costa-de-Oliveira S, Palmeira-de-Oliveira A. Species distribution and antifungal susceptibility profiles of isolates from women with nonrecurrent and recurrent vulvovaginal candidiasis. Microbial Drug Resistance. 2021;27(8):1087-95.
9. Favour EE, Oroboghae OH, Pius OO, Gidion EE. Epidemiology of Oral Candida Infection among People Living With HIV/AIDS (PLWHA) in Bida, Niger State, Nigeria. Sokoto Journal of Medical Laboratory Science. 2017;2(3).
10. Hu L, He C, Zhao C, Chen X, Hua H, Yan Z. Characterization of oral candidiasis and the Candida species profile in patients with oral mucosal diseases. Microbial pathogenesis. 2019 Sep 1;134:103575.
11. Akamike IC, Okedo-Alex IN, Agu AP, Alo C, Ogbonnaya LU. Knowledge and adherence to isoniazid preventive therapy among people living with HIV in multilevel health facilities in South-East, Nigeria: baseline findings from a quasi-experimental study. Pan African Medical Journal. 2020;36(1).1-11
12. Neppelenbroek KH, Seó RS, Urban VM, Silva S, Dovigo LN, Jorge JH, Campanha NH. Identification of Candida species in the clinical laboratory: a review of conventional, commercial, and molecular techniques. Oral diseases. 2014;20(4):329-44.
13. Cheesbrough, M. (2010). District laboratory practice in tropical countries. 2nd edition.
14. Clinical Laboratory Standard Institute (CLSI) Performance Standards for antimicrobial susceptibility testing. Twentieth information supplement (M100 – S20). 9th edition. Clinical Laboratory Standard Institute, Wayne. 2010:1- 253.
15. Zymo Research. Quick-DNA Fungal/Bacterial Kits. 2025.

Print ISSN: 0189-9287 Online ISSN: 2992-345X

https://www.zymoresearch.com/collections/quick-dna-fungal-bacterial-kits?srsltid=AfmBOooYh0pmhgPkohsksLU09ce8UFDfO-NWF17Ib3vmIT7Bz-kIXyC9
16. Yamamoto N, Dannemiller KC, Bibby K, Peccia J. Identification accuracy and diversity reproducibility associated with internal transcribed spacer-based fungal taxonomic library preparation. Environmental microbiology. 2014;16(9):2764-76.
17. Zuo Y, Verheecke-Vaessen C, Molitor C, Medina A, Magan N, Mohareb F. De novo genome assembly and functional annotation for Fusarium langsethiae. BMC genomics. 2022;23(1):158.
18. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS computational biology. 2017 Jun 8;13(6):e1005595.
19. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013;29(8):1072-5.
20. Liu B, Zheng D, Jin Q, Chen L, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic acids research. 2019;47(D1):D687-92.
21. Alcock BP, Raphenya AR, Lau TT, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen AL, Cheng AA, Liu S, Min SY. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic acids research. 2020;48(D1):D517-25.
22. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D. PATRIC, the bacterial bioinformatics database and analysis resource. Nucleic acids research. 2014;42(D1):D581-91.
23. Hirayama T, Miyazaki T, Ito Y, Wakayama M, Shibuya K, Yamashita K, Takazono T, Saijo T, Shimamura S, Yamamoto K, Imamura Y. Virulence assessment of six major pathogenic Candida species in the mouse model of invasive candidiasis caused by fungal translocation. Scientific reports. 2020;10(1):3814.
24. Shadkchan Y, Shemesh E, Mirelman D, Miron T, Rabinkov A, Wilchek M, Osherov N. Efficacy of allicin, the reactive molecule of garlic, in inhibiting Aspergillus spp. in vitro, and in a murine model of disseminated aspergillosis. Journal of Antimicrobial Chemotherapy. 2004 May 1;53(5):832-6.
25. Software. "Tableau Desktop." Tableau Software. 2020. https://www.tableau.com
26. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic acids research. 2015;43(W1):W566-70.
27. Van Rossum, G., & Drake, F. L. Python 3 Reference Manual. CreateSpace, 2009. https://www.python.org
28. Mannweiler O, Pinto-Carbó M, Lardi M, Agnoli K, Eberl L. Investigation of Burkholderia cepacia complex methylomes via single-molecule, real-time sequencing and mutant analysis. Journal of Bacteriology. 2021;203(12):10-128.
29. Kumar A, Francis A, Hans S, Thakur A. Unravelling Drug Resistance in Candida Species: Genetic, Biofilm, Transcriptional, and Epigenetic Perspectives. InRecent Advances in Human Fungal Diseases: Progress and Prospects 2024:203-237).
30. Garcia-Vello P, Di Lorenzo F, Zucchetta D, Zamyatina A, De Castro C, Molinaro A. Lipopolysaccharide lipid A: A promising molecule for new immunity-based therapies and antibiotics. Pharmacology & therapeutics. 2022; 230:107970.
31. Anderson MZ, Saha A, Haseeb A, Bennett RJ. A chromosome 4 trisomy contributes to increased fluconazole resistance in a clinical isolate of Candida albicans. Microbiology. 2017;163(6):856-65.
32. Sanglard D, Coste AT. Activity of isavuconazole and other azoles against Candida clinical isolates and yeast model systems with known azole resistance mechanisms. Antimicrobial agents and chemotherapy. 2016;60(1):229-38.
33. Oliveira F, Lima T, Correia A, Silva AM, Soares C, Morais S, Weißelberg S, Vilanova M, Rohde H, Cerca N. Siderophore-mediated iron acquisition plays a critical role in biofilm formation and survival of Staphylococcus epidermidis within the host. Frontiers in Medicine. 2021 Dec 24; 8:799227.
34. Geiger CJ, Wong GC, O'Toole GA. A bacterial sense of touch: T4P retraction motor as a means of surface sensing by Pseudomonas aeruginosa PA14. Journal of Bacteriology. 2024 Jul 25; 206(7):e00442-23.
35. Nishimoto AT, Zhang Q, Hazlett B, Morschhäuser J, Rogers PD. Contribution of clinically derived mutations in the gene encoding the zinc cluster transcription factor Mrr2 to fluconazole antifungal resistance and CDR1 expression in Candida albicans. Antimicrobial Agents and Chemotherapy. 2019 May; 63(5):10-128.
36. Staniszewska M. Virulence factors in Candida species. Current Protein and Peptide Science. 2020;21(3):313-23.

Print ISSN: 0189-9287 Online ISSN: 2992-345X